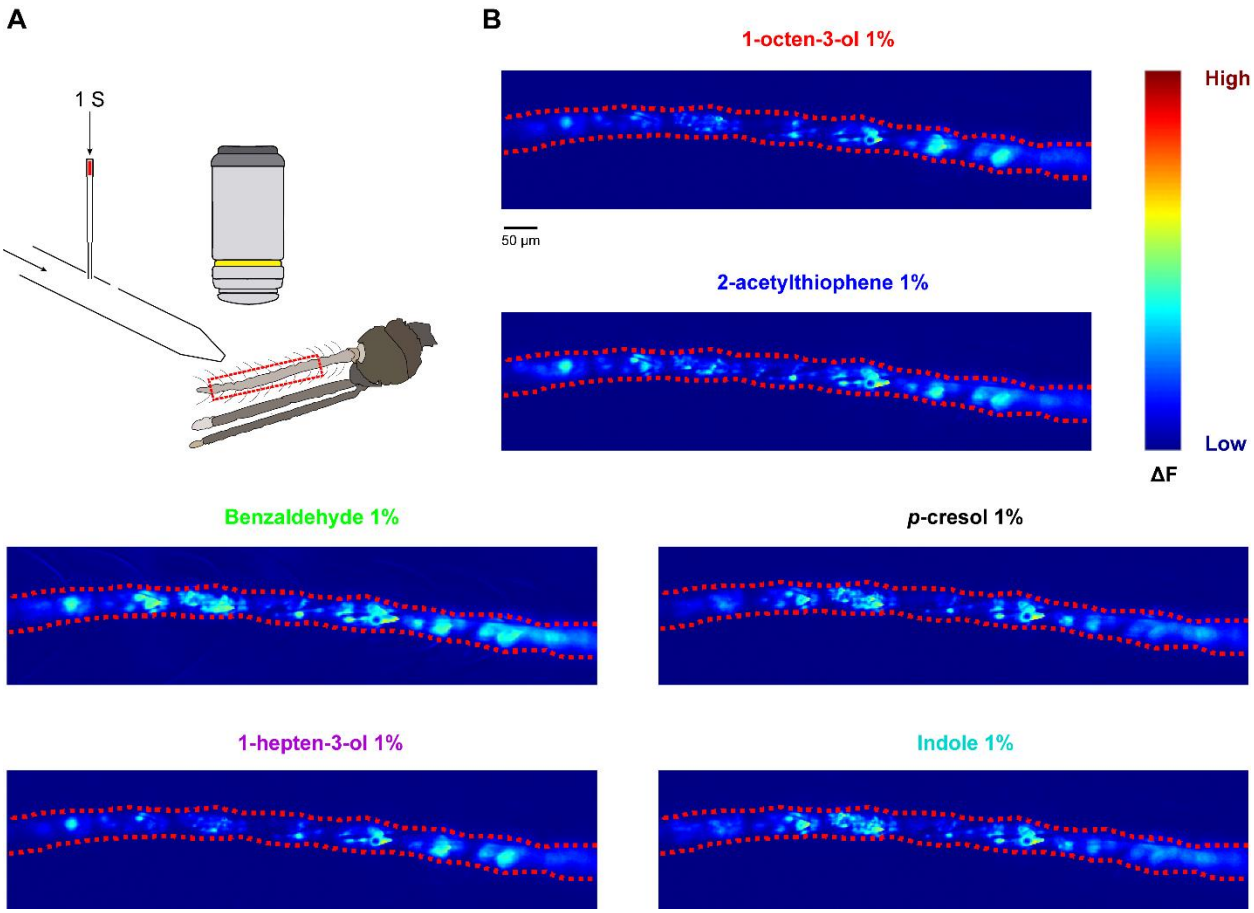

Figure S1. ~~Related to Figure 1.~~ **OR ligands globally activate *An. coluzzii* antennal Orco expressing olfactory neurons.** Related to Figure 1. **A,** Schematic of the calcium imaging setup. A 10x microscope objective is used to image most of the antenna at a time (~ 9/12 olfactory antennal segments outlined in dashed red rectangles). Arrows indicate the direction of air flow (continuous air, and 1 s air pulse). **B,** Example heatmaps of the responses towards each OR ligand at 1% (dashed red lines indicate the borders of the antennae). ΔF values are arbitrary units.

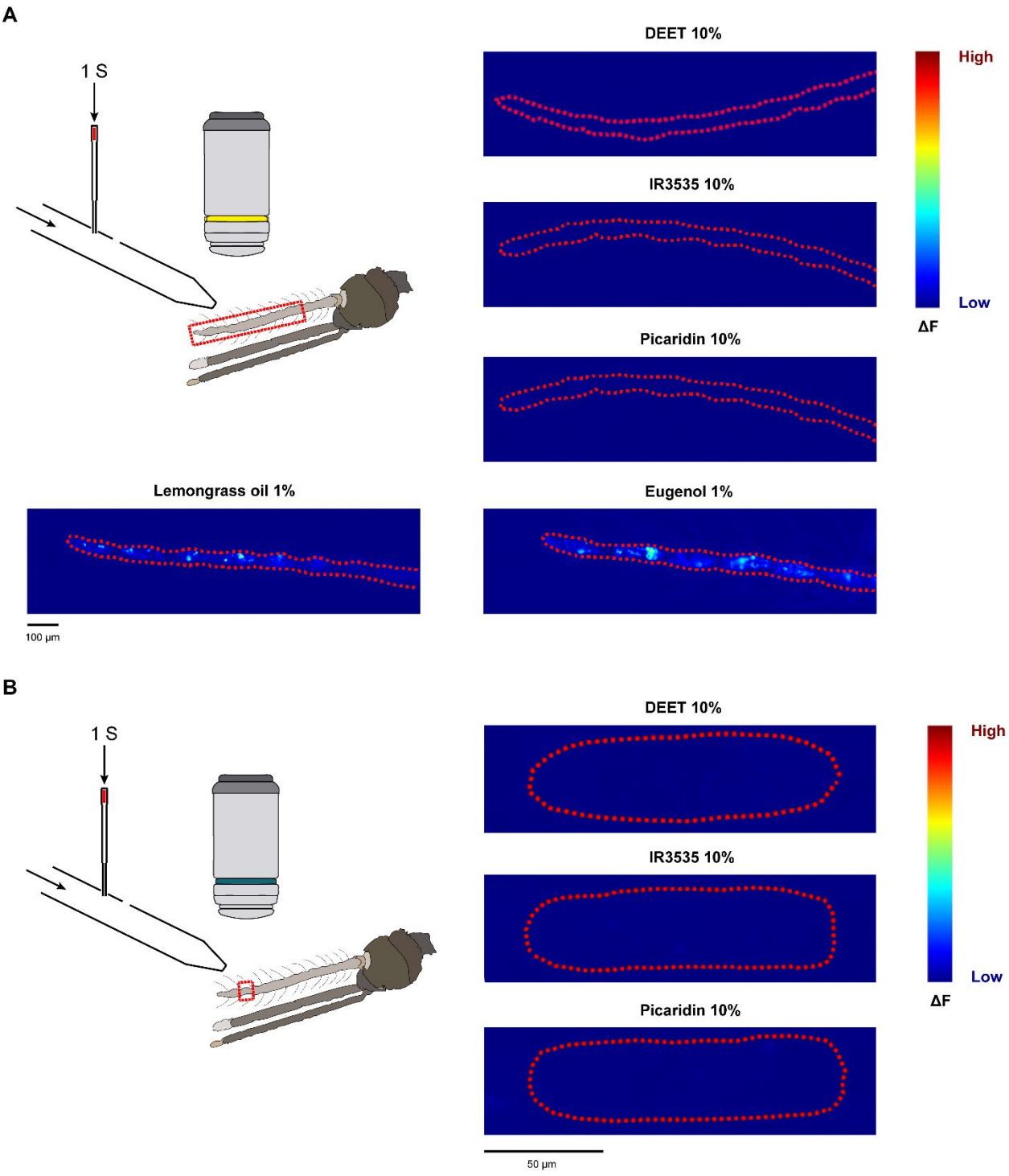

Figure S2. ~~Related to Figure 2.~~ Natural repellents, but not synthetic repellents, strongly activate *Anopheles* olfactory neurons. Related to Figure 2. **A,** Example heatmaps showing responses across most of the antenna (~ 9 segments, dashed red

line) towards 10% DEET, 10% IR3535, and 10% picaridin, and towards 1% of lemongrass oil and 1% eugenol. **B,** Example heatmaps showing higher magnification responses at the 11th antennal segment (dashed red line) towards 10% DEET, 10% IR3535, and 10% picaridin.

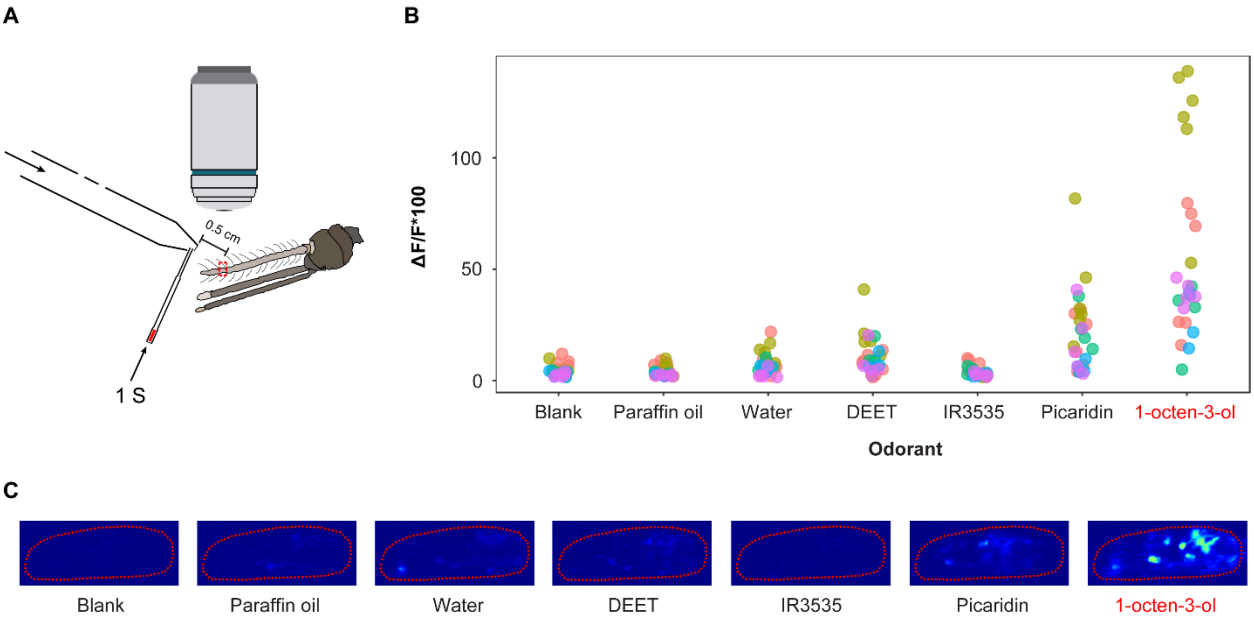

Figure S3. ~~Related to Figure 2.~~ **Close range odor stimulation of the antenna.** Related to Figure 2. **A,** Schematic of the calcium imaging setup with the stimulus Pasteur pipette inserted in a hole at the tip of the long pipette used for continuous air. The stimulus Pasteur pipette is at a 0.5 cm distance from the mosquito antenna. **B,** Responses towards DEET, IR3535, picaridin at 100% in comparison with blank, paraffin oil, water, and 1-octen-3-ol (1%). Each dot represents one neuron, and dots with the same colors represent neurons from the same animal (n=5 animals). Neurons that responded to at least one odorant were plotted (including their responses to all the other odorants). **C,** Example heatmaps for the responses in B.

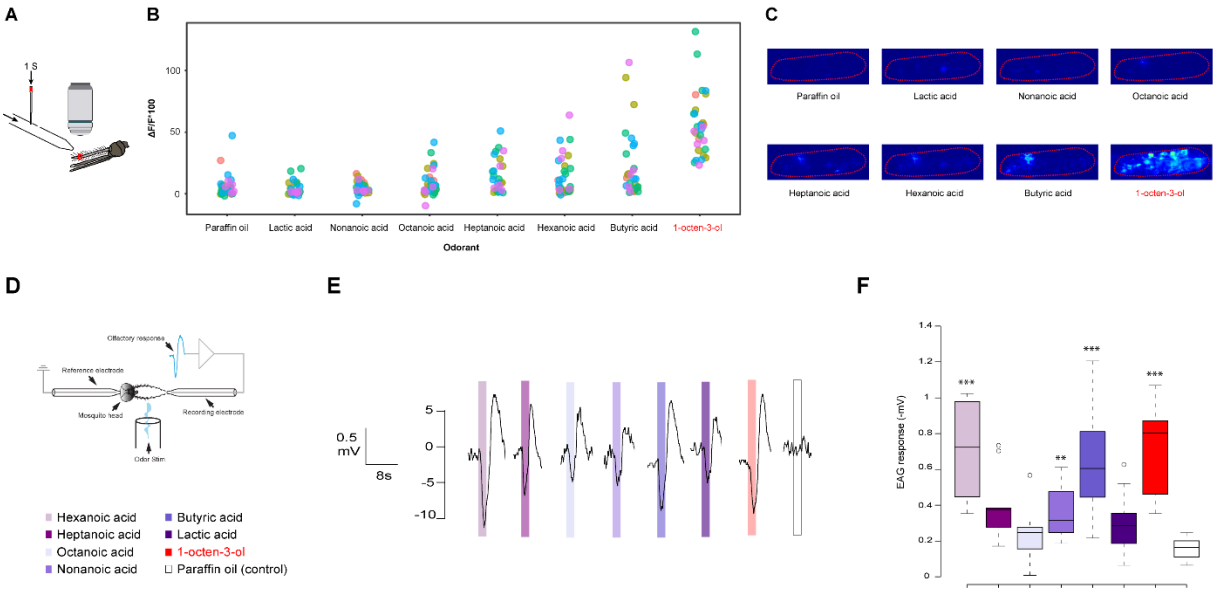

Figure S4. ~~Related to Figure 3.~~ Electroantennogram (EAG) can detect non-Orco neuron responses. Related to Figure 3. **A,** Schematic of the calcium imaging setup**. B,** Responses (ΔF/F*100) to different acids. Dots with the same colors represent neurons from the same animal (n=5 animals). **C,** Example heatmaps of the responses to acids. **D,** Schematic of the EAG setup. **E,** Representative EAG traces for the tested odorants. The colored bar represents the pulse. Note the typical EAG shape of the signal (deflection first) as well as the absence of response to the paraffin oil control. **F,** Boxplots of the EAG responses to different acids. The bar inside the box represents the median while the upper and lower parts of the box represent the 25th and 75th percentiles of the data. Circles represent outliers. N = 10 females. 1-octen-3-ol and paraffin oil were used as controls. Asterisks indicate responses that were significantly different than the paraffin oil response (Pairwise Wilcoxon Rank Sum test with a Bonferroni correction), hexanoic acid, butyric acid, and 1-octen-3-ol ($P < 0.01$), nonanoic acid ($P = 0.02$).

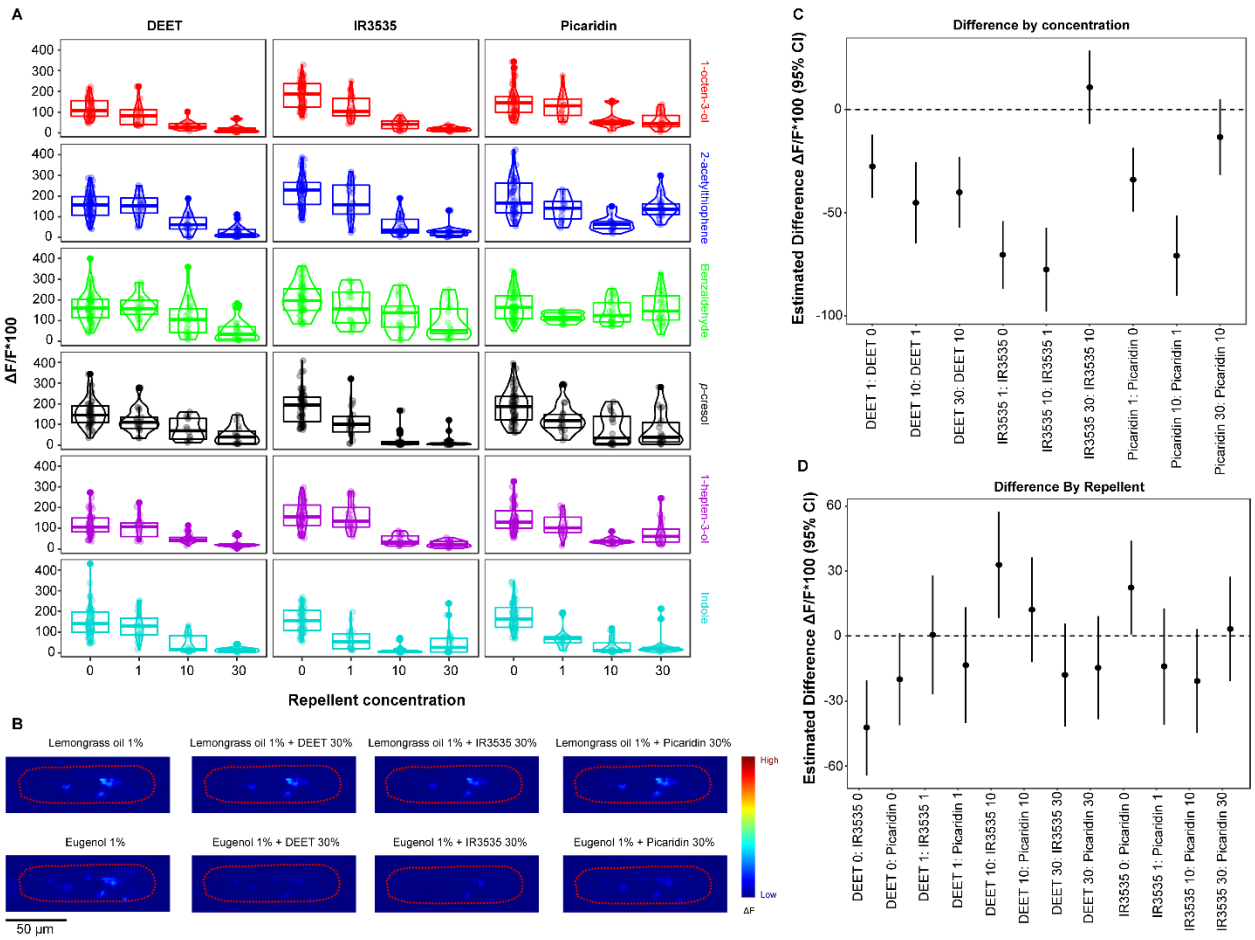

Figure S5. ~~Related to Figure 4.~~ DEET, IR3535, and picaridin mask the olfactory responses towards odorants. Related to Figure 4. **A,** Box plots (median and 25th-75th percentiles) representing raw data for the olfactory responses towards mixtures of the six OR ligands at 1% with repellents (DEET, IR3535, and picaridin) at 0% (OR ligand alone), 1%, 10%, and 30% concentrations (n=15 animals for each condition of 0% repellent, n=5 animals for all other conditions, 1-7 responding neurons/animal, each dot represents a responding neuron). **B,** Example heatmaps of the responses towards 1% of activator repellents (lemongrass oil and eugenol) and their mixtures with 30% DEET, 30% IR3535, or 30% picaridin (n=5 animals for each condition, 2-4 responding neurons/animal). **C,** LME contrasts estimates (mean and 95% Wald CI) between subsequent concentrations

of the same repellent. **D,** LME contrasts estimates (mean and 95% Wald CI) between different repellents at the same concentration. For C and D, CI intersecting with the dashed 0 line indicate non-significant difference ($P > 0.05$, LME model with Wald approximation).

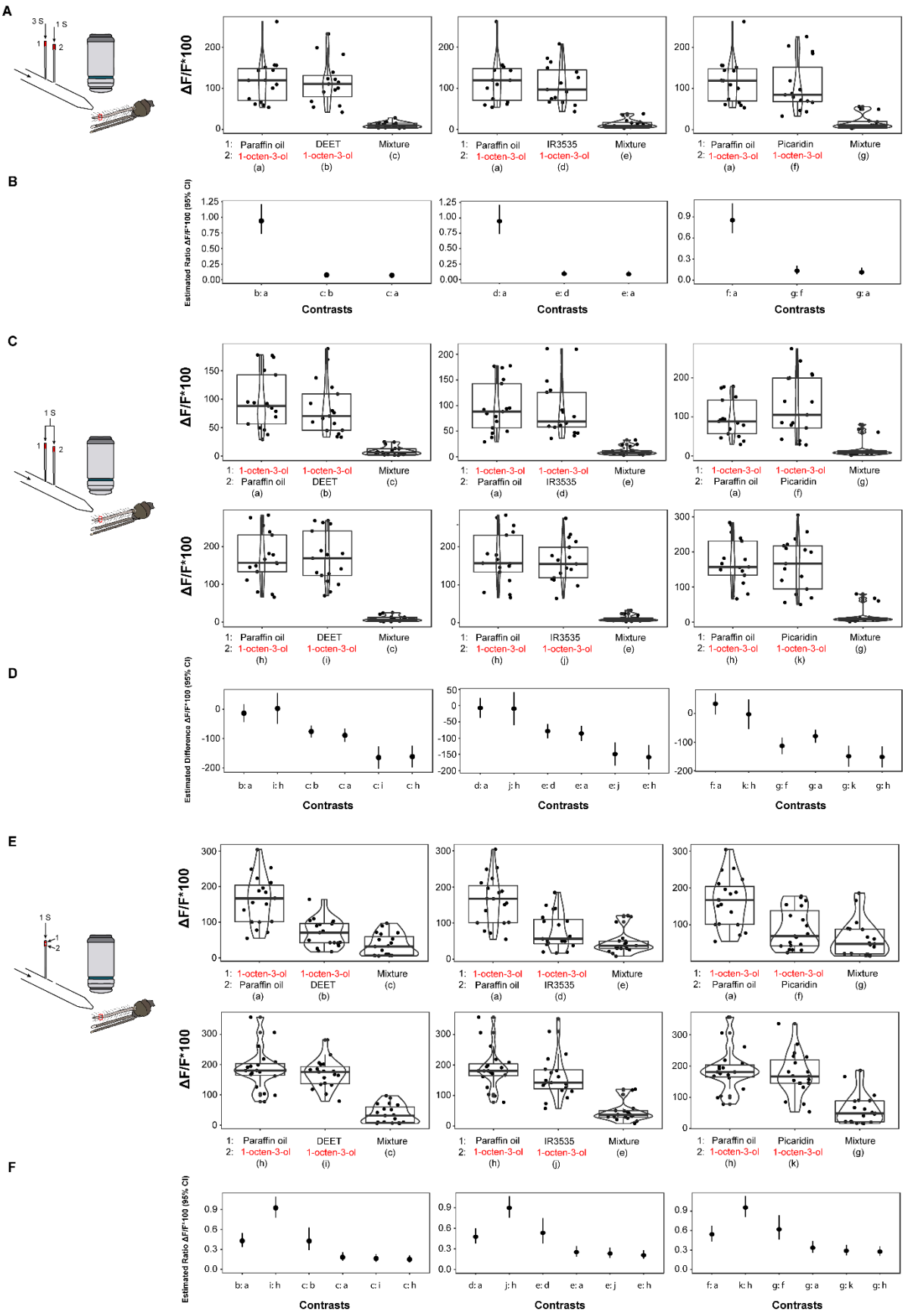
A
B
C
D
E
F
3 S
1 S
1
2
ΔF/F*100
Estimated Ratio ΔF/F*100 (95% CI)
Estimated Difference ΔF/F*100 (95% CI)
Contrasts
1: Paraffin oil
2: 1-octen-3-ol
(a)
DEET
(b)
Mixture
(c)
IR3535
(d)
(e)
Picaridin
(f)
(g)
1: 1-octen-3-ol
2: Paraffin oil
DEET
(h)
(i)
IR3535
(j)
Picaridin
(k)
b: a
c: b
c: a
d: a
e: d
e: a
f: a
g: f
g: a
i: h
c: i
c: h
j: h
e: j
e: h
k: h
g: k
g: h

Figure S6. ~~Related to Figure 5.~~ Olfactory responses to OR ligands are decreased when mixed with synthetic repellents. Related to Figure 5. **A,** Box plots (median and 25th-75th percentiles) representing raw data for the responses towards a 1 s pulse of 1% 1-octen-3-ol occurring during the last second of a 3 s pulse of paraffin oil, 30% DEET, 30% IR3535, or 30% picaridin, compared to the response towards physical mixtures of 1% 1-octen-3-ol with 30% of DEET, 30% IR3535, or 30% picaridin. The numbers next to odorant names indicate the position of the Pasteur pipette that contains that odorant in the schematic. **B,** LME contrasts estimates (mean and 95% Wald CI) between odorant conditions in A (letters on the X-axis correspond to the letters in A to indicate odorant conditions). **C,** Box plots (median and 25th-75th percentiles) representing raw data for the responses towards a 1 s pulse of 1% 1-octen-3-ol in the first position or the second position simultaneously delivered with a 3 s pulse of paraffin oil, 30% DEET, 30% IR3535, or 30% picaridin, compared to the response towards physical mixtures of 1% 1-octen-3-ol with 30% of DEET, 30% IR3535, or 30% picaridin. **D,** LME contrasts estimates (mean and 95% Wald CI) between odorant conditions in C (letters on the X-axis correspond to letters in C to indicate odorant conditions). **E,** Box plots (median and 25th-75th percentiles) representing raw data for the responses towards a 1 s pulse of 1% 1-octen-3-ol when applied on the upper filter paper or the lower filter paper with paraffin oil, 30% DEET, 30% IR3535, or 30% picaridin in the same Pasteur pipette, compared to the response towards physical mixtures of 1% 1-octen-3-ol with 30% of DEET, 30% IR3535, or 30% picaridin. **F,** LME contrasts estimates (mean and 95% Wald CI) between odorant conditions in E (letters on the X-axis correspond to letters in E to indicate odorant conditions). Contrasts in B and F come from a log transformed model, representing a ratio of geometric means:

e.g. a value of 1 indicates no difference, a value of 0.5 indicates a 50% reduction, and a value of 1.5 indicates a 50% increase. Contrasts in D come from a model fit to the raw data; CI intersecting with the dashed 0 line indicate non-significant difference. For A, C, E, n=5 animals for each condition, 1-6 responding neurons/animal, each dot represents a responding neuron.